510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K181156

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Panther Fusion GBS Assay

C. Measurand:

sip and *cfb* gene sequence of *Streptococcus agalactiae* chromosome (Group B *Streptococcus*)

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) test

E. Applicant:

Diagenode

F. Proprietary and Established Names:

Panther Fusion GBS Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3740: *Streptococcus* spp. serological reagents

2. Classification:

   Class I (non-exempt)

3. Product code:

   NJR: Nucleic Acid Amplification Assay System, Group B Streptococcus, Direct Specimen Test
   OOI: Real-Time Nucleic Acid Amplification System

4. Panel:

   83-Microbiology

H. Intended Use:

1. Intended use(s):
   The Panther Fusion GBS assay is an automated qualitative *in vitro* diagnostic test utilizing real-time PCR for detection of Group B *Streptococcus* DNA from either Lim or Carrot enrichment broth cultures of vaginal/rectal swabs from antepartum women following 18-24 hours incubation.

   This test is performed on the Hologic Panther Fusion system and is intended to aid in determining the GBS colonization status of antepartum women. This assay does not diagnose or monitor treatment for GBS infections. The Panther Fusion GBS assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic use.

   For prescription use only.

4. Special instrument requirements:

   The Panther Fusion GBS Assay is to be performed on the Hologic Panther Fusion system.

I. Device Description:

The Panther Fusion GBS Assay is a single use real-time PCR test for the qualitative detection of Group B *Streptococcus* (GBS) DNA from a vaginal/rectal swab specimen following 18-24 hours incubation in selective enrichment broth culture (Lim Broth and Carrot Broth). The Panther Fusion GBS Assay involves the following steps: Sample lysis, nucleic acid capture and elution, and multiplex real-time-PCR where analytes (when present) are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution takes place in a single tube. The eluate is transferred to a reaction tube containing the assay reagents. Real-time PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Assay Components

The reagents required to perform the Panther Fusion GBS Assay are packed together in the Panther Fusion GBS Assay box. A description of the components that are required to perform the Panther Fusion GBS Assay are detailed in **Table 1**.

Table 1: Reagents Required to Perform the Panther Fusion GBS Assay*

Box	Components
1	Panther Fusion GBS Cartridges
2	Panther Fusion Extraction Reagent- - Panther Fusion Capture Reagent - Panther Fusion Enhancer Reagent
3	Panther Fusion Internal Control
4	Panther Fusion Elution Buffer
5	Panther Fusion Reconstitution Buffer I
6	Panther Fusion Oil
7	Panther Fusion GBS Assay Controls - Panther Fusion GBS Assay Positive Control - Panther Fusion GBS Assay Negative Control

**See Panther Fusion GBS package insert for a complete list of Materials Required and Available Separately*.

Instrumentation

The Panther Fusion system is an integrated hardware and software system that together with the Panther Fusion GBS Assay automates all the steps necessary to perform the assay. The Panther Fusion system integrates Hologic's commercialized Panther instrument system with an add-on sidecar, the Panther Fusion module, which extends the functionality of the Panther system by increasing the assay processing capabilities. The Panther Fusion module includes instrument hardware and software and can be installed on existing Panther instruments or ordered with new Panther instruments.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Cepheid Xpert GBS LB Assay

2. Predicate 510(k) number(s):

   K121539

3. Comparison with predicate:

Similarities		
Item	**Panther Fusion GBS Assay (K181156)**	**Cepheid Xpert GBS LB Assay (K121539)**
Intended Use	The Panther Fusion GBS assay is an automated qualitative *in vitro* diagnostic test utilizing	The Cepheid Xpert GBS LB Assay, performed on the GeneXpert Instrument Systems,

Similarities		
Item	**Panther Fusion GBS Assay (K181156)**	**Cepheid Xpert GBS LB Assay (K121539)**
	real-time PCR for detection of Group B *Streptococcus* DNA from either Lim or Carrot enrichment broth cultures of vaginal/rectal swabs from antepartum women following 18-24 hours incubation. This test is performed on the Hologic Panther Fusion system and is intended to aid in determining the GBS colonization status of antepartum women. This assay does not diagnose or monitor treatment for GBS infections. The Panther Fusion GBS assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.	is a qualitative *in vitro* diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens, using fully automated real-time polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA. Xpert GBS LB Assay testing is indicated as an aid in determining GBS colonization status in antepartum women. The Xpert GBS LB Assay is used for antepartum testing on enriched Lim broth cultures of vaginal/rectal swabs after 18-24 hours of incubation. The Xpert GBS LB assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin allergic women.
Technology Principle of Operation	Fully automated nucleic acid amplification, detection and result interpretation	Same
Analyte	Genomic DNA	Same
Organism Detected	Group B *Streptococcus*	Same
Patient Population	Antepartum women	Same
Specimen Types	From 18-24 hour enriched-media cultures of vaginal/rectal swab	Same
Assay Format	Amplification: Real-time PCR Detection: Fluorogenic target-specific hybridization	Same

Differences		
Item	**Panther Fusion GBS Assay (K181156)**	**Cepheid Xpert GBS LB Assay (K121539)**
Platform	Panther Fusion System	GeneXpert Dx System GeneXpert Infinity-48 System GeneXpert Infinity-80 System
DNA Target Sequence	*sip* and *cfb* genes	3'untranslated region of the *cfb* gene
Specimen Enrichment	Lim Broth and Carrot Broth	Lim Broth
Time to Obtain Results	≤ 2.5 hours total after sample loading on the Panther Fusion system	≤ 55 minutes total after sample addition to the cartridge

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The Panther Fusion GBS Assay is a single use real-time PCR test. The assay is performed on the automated Panther Fusion system which includes DNA extraction, target amplification and detection of targets from enrichment broth. The PCR reagents are packaged as ready to use lyophilized reagents in cartridges that can be loaded onto the Panther Fusion system.

Sample Collection and Enrichment: Prior to testing on the Panther Fusion system, enriched specimens are transferred to a tube containing specimen transport media that lyses the cells, releases target nucleic acids, and protects them from degradation during storage.

Nucleic Acid Capture and Elution: Magnetic particles contained in Panther Fusion Capture Reagent mediate the nucleic acid capture by hybridizing to total nucleic acid in the test specimen. The capture nucleic acids are then separated from residual specimen matrix in a magnetic field by a series of wash steps with a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a Panther Fusion Elution Buffer.

Elution Transfer and PCR: Eluted nucleic acid is transferred to a Panther Fusion Reaction tube containing reconstituted mastermix. PCR-based target amplification subsequently occurs with target-specific forward and reverse primers, generating a fluorescence signal. Each PCR test includes two sets of primers and probes for the amplification and detection of GBS DNA target sequences. An Internal Control (IC), which serves as a control for monitoring the entire testing process including DNA extraction, amplification and detection is included in each test. Positive and negative run controls are also provided as part of the assay kit.

The Panther Fusion system is configured to require that assay controls run at an administrator-specified interval up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing. During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Results Interpretation: The Panther Fusion GBS Software computes a cycle threshold result (Ct) to qualitatively determine the presence of the analyte and compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analytes. The analyte targets and corresponding fluorescent channels used in the Panther Fusion GBS Assay are summarized in **Table 2** below.

Table 2: Analytes and Detection Channels

Analyte	Gene Targeted	Instrument Channel
GBS	*sip* and *cfb*	FAM
Internal Control (IC)	Not Applicable	RED677

Target amplification takes place on the Panther Fusion thermocycler with concurrent real-time fluorescent detection. The results are displayed on the screen at the end of the assay, which can be printed, transferred, and stored by the end user (**Table 3**).

Table 3. Possible Results Reported in a Valid Run with Result Interpretations

GBS Result	IC Result	Interpretation
Negative	Valid	GBS DNA not detected.
Positive	Valid	GBS DNA detected.
Invalid	Invalid	Invalid. There was an error in the generation of the result. Retest sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision Study

A Precision Study was conducted with a seven-member panel to evaluate the within-site variability over 12 days with three different reagent lots on one Panther Fusion system. Panel members were tested in triplicate on five separate runs per day. Three different GBS strains (serotype III, serotype V, and a non-hemolytic) were used to prepare panel members, which included true negative, low positive (1-2X LoD), and moderate positive (3X LoD) samples. Panel members were prepared in Lim Broth matrix spiked with GBS. For each run, a positive and negative external control were processed in addition to the panel samples. A total of 1260 samples were prepared for the study, where 180 samples per panel member were tested. The percent agreement with the expected result for each low positive, moderate positive, and true negative panel member was $\geq$95% as shown in **Table 4**. **Table 5** shows a summary of the coefficient of variation (%CV) and standard deviation (SD) for Ct values. All values met the acceptance criteria for the study.

Table 4. GBS Positivity Rate and % Agreement to Expected Result

Panel Member	% Positive (Pos n/valid n)	% Agreement (95% CI)
GBS III Low Positive (1-2X LoD)	100% (180/180)	100% (97.9-100)
GBS III Moderate Positive (3X LoD)	100% (180/180)	100% (97.9-100)
GBS V Low Positive (1-2X LoD)	100% (180/180)	100% (97.9-100)
GBS V Moderate Positive (3X LoD)	100% (180/180)	100% (97.9-100)
GBS NH Low Positive (1-2X LoD)	100% (180/180)	100% (97.9-100)
GBS NH Moderate Positive (3X LoD)	100% (180/180)	100% (97.9-100)
Negative	0% (0/180)	100% (97.9-100)

CI= confidence interval, LoD= limit of detection, NH= non-hemolytic

Table 5. Summary of the SD and %CV for the Ct values in the Within-Site Precision Study

Panel Member	*Mean Ct*	*Between Reagents Lots*		*Between Operators*		*Between Days*		*Between Runs*		*Within Runs*		*Total*	
		SD	*CV(%)*	*SD*	*CV(%)*	*SD*	*CV(%)*	*SD*	*CV(%)*	*SD*	*CV(%)*	*SD*	*CV(%)*
GBS III 1-2X LoD	*36.3*	*0.06*	*0.16%*	*0.16*	*0.45%*	*0.11*	*0.31%*	*0.33*	*0.91%*	*0.43*	*1.18%*	*0.53*	*1.46%*
GBS III 3X LoD	*35.4*	*0.17*	*0.48%*	*0.12*	*0.35%*	*0.13*	*0.36%*	*0.34*	*0.95%*	*0.32*	*0.90%*	*0.43*	*1.22%*
GBS V 1-2X LoD	*36.4*	*0.13*	*0.37%*	*0.13*	*0.35%*	*0.17*	*0.46%*	*0.29*	*0.78%*	*0.50*	*1.36%*	*0.55*	*1.51%*
GBS V 3X LoD	*35.4*	*0.13*	*0.38%*	*0.11*	*0.31%*	*0.12*	*0.34%*	*0.28*	*0.79%*	*0.41*	*1.14%*	*0.46*	*1.31%*
GBS NH 1-2X LoD	*35.7*	*0.23*	*0.65%*	*0.12*	*0.35%*	*0.14*	*0.39%*	*0.31*	*0.86%*	*0.38*	*1.06%*	*0.46*	*1.28%*
GBS NH 3X LoD	*34.8*	*0.19*	*0.55%*	*0.04*	*0.12%*	*0.10*	*0.29%*	*0.28*	*0.81%*	*0.29*	*0.84%*	*0.40*	*1.14%*
Negative	*31.5*	*0.24*	*0.77%*	*0.08*	*0.24%*	*0.14*	*0.43%*	*0.32*	*1.03%*	*0.27*	*0.86%*	*0.41*	*1.32%*

Reproducibility

The reproducibility of the Panther Fusion GBS Assay was evaluated by testing the assay on three Panther Fusion instruments with two operators at each of the three external sites for five days. Testing was performed using one lot of assay reagents and six operators (two at each site). A blinded reproducibility panel was prepared similar to the Precision Study panel. Across three sites, a total of 90 replicates were tested per panel member with the Panther Fusion GBS Assay. The percent agreement with the expected result was >95% for GBS samples tested at low positive and moderate positive concentrations. The true negatives yielded the expected results 100% of the time. A total of thirty positive and negative controls also yielded the expected result. Twelve invalid samples occurred as a result of a hardware failure. All of the invalid samples were re-tested per the labeled instructions and produced valid results. The results from the site-to-site Reproducibility Study for the Panther Fusion GBS Assay are presented in **Table 6** below.

Table 6. Summary of Panel Member Agreement with Expected Result

Panels			Expected Result	Agreement with Expected Result	
Description	**Composition**	**Concentration (CFU/ml)**	**GBS**	**N**	**% (95% CI)**
GBS III Low Positive	1-2X LoD	262	+	90/90	100% (95.9-100%)
GBS III Moderate Positive	3X LoD	504	+	90/90	100% (95.9-100%)
GBS V Low Positive	1-2X LoD	188	+	89/90	98.9% (94.0-99.8%)
GBS V Moderate Positive	3X LoD	367	+	90/90	100% (95.9-100%)
GBS NH Low Positive	1-2X LoD	523	+	90/90	100% (95.9-100%)
GBS NH Moderate Positive	3X LoD	900	+	90/90	100% (95.9-100%)
Neg.	Negative	N/A	-	90/90	100% (95.9-100%)

The Reproducibility/Precision Studies are acceptable.

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

External Controls
One replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active assay cartridge lot has expired.

- *Positive Control:* The positive run control consisted of inactivated whole cell GBS bacteria stock acquired from Zeptometrix and diluted in Specimen Transport Medium.
- *Negative Control:* The negative run control consisted of Specimen Transport Medium.

For all clinical sites combined, a total of 28 positive and negative Run Controls were tested during the study. There were no invalid runs, and all controls produced the expected result.

Reagent Stability

Shelf-life and On-board Stability
A real-time stability study is currently in process to establish the intended shelf-life of

assay reagents. Components were manufactured at different times and stored at the recommended temperature and conditions. Initial product dating for Fusion Oil, Elution Buffer, Recon Buffer and the Negative Control was established by the Panther Fusion Flu A/B/RSV Stability Studies (K171963).

The Panther Fusion GBS Assay performance will be evaluated for each storage condition at baseline and at different timepoints until the last designed time points are reached. The following table (**Table 7** below) shows the current storage and handling conditions for the assay that are supported.

Table 7. Reagent Storage Conditions.

Platform	Reagent	Unopened Storage	On board/ Open Stability	Opened Storage
Panther Fusion GBS Assay	Panther Fusion GBS Assay	2°C to 8°C	60 days	2°C to 8°C
	Panther Fusion Capture Reagent-X	15°C to 30°C	30 days	15°C to 30°C
	Panther Fusion Enhancer Reagent-X	15°C to 30°C	30 days	15°C to 30°C
	Panther Fusion Internal Control-X	2°C to 8°C	(in wFCR-X)	Not Applicable
	Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
	Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
	Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
	Panther Fusion GBS Positive Control	2°C to 8°C	Single use vial	N/A-single use
	Panther Fusion Negative Control	2°C to 8°C	Single use vial	N/A-single use

Shipping Stability

One lot of reagents and controls for the Panther Fusion GBS Assay were tested with three panel members in this study. Two positive panels members consisted of *sip* or *cfb* plasmid diluted in Specimen Transport Medium, and the negative panel member consisted of Specimen Transport Medium alone. Shipping stability was evaluated by exposing reagents and controls to five cycles of temperature variation for up to 280 hours. Each cycle consisted of eight hours of exposure to extremely low temperatures (< -40 °C) followed by eight hours of exposure to high temperatures (55°C) these exposure phases were interspersed with storage at room temperature for 16 hours. The performance of reagents and controls under controlled ambient shipping conditions (15°C to 30°C) was also assessed as reference. Reagents performances was evaluated at the end of each cycle by functional testing triplicates of the three-member panel (two positives and one negative). At each timepoint and for each shipping condition tested, all samples gave expected results demonstrating the stability of Panther Fusion GBS Assay reagents and controls at room temperature and extreme shipping conditions for up to 288 hours.

Sample Stability

A study was conducted to establish stability for samples after collection, enrichment in selective broth (Lim Broth and Carrot Broth), and dilution in Specimen Transport Medium for Panther Fusion processing.

- *Swab Sample Stability*

  The Swab Sample Stability Study was conducted with samples in Amies transport medium at 15°C and 30°C for up to 48 hours. To simulate vaginal/rectal swab specimen collection and transportation conditions from patients to testing lab, Amies was spiked with GBS serotype III at different concentrations: 10X LoD, 3X LoD and 50 CFU/swab. Amies samples were stored at room temperature (15°C and 30°C) and tested at baseline (T=0) and at different time points for up to 48 hours with six hours interval between the time points. At each storage time point, Amies samples were enriched either in Lim Broth or in Carrot Broth for 18-24 hours and then processed for the Panther Fusion GBS Assay. Eight samples were tested for each time point and storage condition. For all time points tested up to 48 hours of storage at 15°C and 30°C, 100% of the swabs spiked with GBS serotype III at 50 CFU/Swab, 3X LoD and 10X LoD gave GBS positive results when tested with the Panther Fusion GBS Assay.

- *Enriched Sample Stability*

  The Enriched Sample Stability Study was conducted with samples enriched in Lim Broth or Carrot Broth at 2°C and 8°C for up to five days, and at 15°C and 30°C for up to 24 hours. Clinical negative Amies medium was use to represent the swab sample. Amies medium was spiked with GBS serotype III at 3X LoD and used to inoculate fresh Lim Broth and Carrot Broth. Cultures were incubated at 35-37°C for 18-24 hours. Separate enrichment broth aliquots were prepared for each time point. Enriched samples where then stored under the following storage conditions:

  - Room temperature (15°C and 30°C) for up to 24 hours
  - Refrigerated temperature (2°C and 8°C) for up to five days

  For enriched cultures stored at room temperature, the test was performed at 0, 6, 12 and 24 hours of storage. For enriched cultures stored at refrigerated temperature, the test was performed at 0, 1, 2, 3, 4 and 5 days of storage. At each storage time point, samples were processed and tested on the Panther Fusion GBS Assay. Eight samples were tested for each time point and storage condition. After 24 hours of storage at room temperatures (15°C and 30°C), 100% of the enriched samples gave GBS positive results when tested with the Panther Fusion GBS Assay. After five days of storage at refrigerated temperatures (2°C and 8°C), 100% of the enriched samples gave GBS positive result when tested with the Panther Fusion GBS Assay.

- *Processed Sample Stability*

  The Processed Sample Stability Study was conducted with enriched samples transferred in Specimen Transport Medium and kept at 2°C and 8°C for up to five

days, as well as 15°C and 30°C for up to 72 hours. Enrichment broths inoculated with GBS serotype III at 3X LoD and 10X LoD were stored for five days at 8°C. After five days of storage for the enriched culture, a processed sample was prepared by adding an aliquot of each enrichment broth in an Aptima Transfer Tube containing Specimen Transport Medium. Samples were then stored under the following conditions:

- Room temperature (15°C and 30°C) for up to 72 hours
- Refrigerated temperature (2°C and 8°C) for up to five days

Eight samples were tested for each time point and storage condition. After 72 hours of storage at room temperatures (15°C and 30°C), 100% of the processed samples gave GBS positive results when tested with the Panther Fusion GBS Assay. After five days of storage at refrigerated temperatures (2°C and 8°C), 100% of the processed samples gave GBS positive result after processing with the Panther Fusion GBS Assay.

Stability claims for samples to be tested with the Panther Fusion GBS Assay are as follows:

- Swab samples are stable up to 48 hours at room temperature (15-30°C)
- Enriched sample in Lim Broth or in Carrot Broth are stable for up to 24 hours at room temperature (15-30°C) and for up to five days at refrigerated temperature (2-8°C).
- The processed samples (enriched culture in Specimen Transport Medium) are stable for up to 72 hours at room temperature (15-30°C) and for up to five days at refrigerated temperature.

d. Detection limit

LoD and Analytical Sensitivity

The LoD of the Panther Fusion GBS Assay for multiple GBS strains was determined by testing serial dilutions of 11 GBS serotypes (Ia, Ib, Ic, II, III, IV, V, VI, VII, VIII, and IX) and one non-hemolytic (NH) isolate in negative clinical Lim Broth matrix. Thirty replicates were tested with each of three reagent lots for a combined total of 90 replicates per serotype and dilution. Probit analysis was performed for each reagent lot with the reported 95% LoD based upon the worst estimate, as shown in **Table 8**. Serotype specific LoD estimates were confirmed by testing an additional 20 replicates with one reagent lot.

Table 8. Limit of Detection

GBS Serotype	95% LoD in CFU/ml (95% CI)
Ia	137.4 (103.7 – 209.7)
Ib	140.5 (100.6 – 234.7)
Ic	136.3 (99.2 – 220.5)
II	179.0 (135.1 – 276.2)
III	168.0 (125.2 – 261.5)

IV	84.0 (63.0 – 130.4)
V	122.3 (92.2 – 186.8)
VI	282.0 (201.9 – 475.8)
VII	250.8 (180.2 – 424.8)
VIII	231.3 (167.3 – 380.9)
IX	301.0 (202.0 – 567.7)
NH	300.2 (212.0 – 523.0)

Broth Equivalence Study

The purpose of this study was to demonstrate equivalence between Lim and Carrot enrichment broths with the Panther Fusion GBS Assay. Three GBS strains (serotype III, serotype V, and a non-hemolytic) were serially diluted in clinical negative matrices of Lim Broth or Carrot Broth to their respective concentrations (at 0.5X, 2X and 5X LoD) based on data obtained during the LoD study. For each type of broth, 30 replicates per strain and concentration as well as 30 replicates of negative matrix were processed. Testing was performed with one reagent lot and one Panther Fusion system. All five attempted runs were valid and the internal control (IC) was detected in each of the 1785 reactions, resulting in respectively a 0% invalid run rate and a 0% internal control invalid rate.

Positivity results for Lim Broth and Carrot Broth are summarized in **Table 9**. The Panther Fusion GBS Assay demonstrated equivalent performance between Lim Broth and Carrot Broth at 2X LoD and 5X LoD concentrations. Sample concentrations below the assay LoD were tested to ensure that the panel concentrations challenged the assay. Enriched specimens are expected to contain target concentrations above the Panther Fusion GBS Assay LoD.

Table 9. Broth Equivalence Study with GBS Spiked with 0.5X, 2X, and 5X LoD Concentrations.

GBS Strain	GBS Positive Calls (%)		
	0.5X LoD	2X LoD	5X LoD
Serotype Ia (Lim)	70%	100%	100%
Serotype Ia (Carrot)	70%	100%	100%
Serotype Ib (Lim)	60%	100%	100%
Serotype Ib (Carrot)	71.43%	100%	100%
Serotype Ic (Lim)	66.67%	100%	100%
Serotype Ic (Carrot)	66.67%	100%	100%
Serotype II	50%	96.67%	100%

(Lim)			
Serotype II (Carrot)	70%	100%	100%
Serotype III (Lim)	76.7%*	96.7%	100%
Serotype III (Carrot)	100%*	100%	100%
Serotype IV (Lim)	40%	100%	100%
Serotype IV (Carrot)	93.33%	100%	100%
Serotype V (Lim)	73.33%*	100%	100%
Serotype V (Carrot)	90%*	100%	100%
Non-hemolytic (Lim)	86.7%*	100%	100%
Non-hemolytic (Carrot)	93.3%*	100%	100%

**Positivity rate above represents one of two sets data collected at 0.5X LoD concentration for these particular strains. The second set of positivity rates obtained were as follows—Serotype III (Lim), 83.3%; Serotype III (Carrot), 80%; Serotype V (Lim),70%; Serotype V (Carrot), 93.3%; Non-hemolytic (Lim), 90%; Non-hemolytic (Carrot), 96.7%.*

e. *Analytical specificity:*

Cross-Reactivity and Microbial Interference

A study was conducted with the Panther Fusion GBS Assay to determine the cross-reactivity of 110 microorganisms (or nucleic acid) representing various non-GBS groups of *Streptococci*, along with other bacteria, protozoa/parasites, yeast/fungi, and viruses common to the urogenital and digestive tract (**Table 10**). Bacteria and yeast were tested at $\geq 10^6$ CFU/ml. Viruses were tested at the at 1x 10^5 PFU/ml, except where noted. To simplify testing, various pools were prepared with five organisms spiked into negative Lim Broth clinical matrix. Organisms were tested in triplicate both with and without GBS (spiked at a concentration 3X the established LoD). None of the tested microorganisms were reported as cross-reactive or interfering with the Panther Fusion GBS Assay.

Table 10. Cross-Reactivity Organism Panel

Bacillus cereus	*Citrobacter freundii*	*Rhodococcus equi*
Yersinia enterocolitica subsp. enterocolitica	*Enterococcus gallinarum*	*Listeria monocytogenes*
Anaerococcus prevotii	*Acinetobacter lwoffii*	*Lactobacillus gasseri*
Propionibacterium acnes	*Pseudomonas aeruginosa*	*Peptoniphilus asaccharolyticus*
Clostridium difficile	*Streptococcus criceti*	*Atopobium vaginae*
Fusobacterium nucleatum	*Haemophilus influenzae*	*Bifidobacterium brevis*
Bifidobacterium adolescentis	*Klebsiella oxytoca*	*Abiotropha defectiva*

Reuter		
Candida albicans (NIH 3147)	*Streptococcus bovis (group D)*	*Anaerococcus tetradius*
Candida glabrata (CBS 138)	*Streptococcus parasanguinis*	*Finegoldia magna*
Candida tropicalis	*Streptococcus equi subsp. equi (group D)*	*Peptostreptococcus anaerobius*
Cryptococcus neoformans*	*Enterococcus durans*	*Anaerococcus lactolyticus*
Klebsiella pneumoniae	*Lactobacillus plantarum*	Human herpesvirus 4 (EBV)*
Proteus mirabilis	*Streptococcus dysgalactiae*	*Bacteroides fragilis*
Alcaligenes faecalis	*Streptococcus constellatus*	*Bordetella pertussis*
Enterobacter aerogenes	*Streptococcus oralis (oral group)*	*Chlamydia trachomatis*
Stenotrophomonas maltophilia	*Bacillus coagulans*	Human herpesvirus 5 (CMV)*
Campylobacter jejuni	*Streptococcus pseudoporcinus*	*Hafnia alvei*
Providencia stuartii	*Streptococcus mitis (oral group)*	*Trichomonas vaginalis**
Micrococcus luteus	*Streptococcus anginosus*	Human immuno-deficiency virus-1 (HIV-1)*
Staphylococcus haemolyticus	*Prevotella oralis*	*Moraxella catarrhalis*
Enterococcus faecalis	*Streptococcus canis*	*Mycoplasma genitalium*
Pseudomonas fluorescens	*Lactobacillus delbrueckii subsp. lactis*	*Prevotella melaninogenica*
Staphylococcus saprophyticus	*Corynebacterium sp (genitalium)*	Rubella Virus*
Proteus vulgaris	*Neisseria gonorrhoeae*	*Serratia marcescens*
*Toxoplasma gondii**	*Streptococcus pneumoniae (oral group)*	*Streptococcus intermedius*
Enterococcus faecium	*Streptococcus mutans (oral group)*	Human Papilloma Virus Type 16 (HPV16)*
Escherichia coli	*Corynebacterium urealyticum*	Hepatitis B Virus*
Enterobacter cloacae	*Lactobacillus reuteri*	Hepatitis C Virus*
Morganella morganii	*Lactobacillus sp.*	Herpes Simplex Virus-1 (HSV-1)*
Shigella flexneri	*Lactobacillus casei*	Herpes Simplex Virus-2 (HSV-2)*
Streptococcus pyogenes (group A)	*Lactobacillus acidophilus*	Human herpesvirus 3 (VZV)*
Streptococcus ratti	*Streptococcus gordonii (oral group)*	*Arcanobacterium pyogenes*
Staphylococcus lugdunensis	*Bulkholderia cepacia*	*Mobiluncus curtisii subsp. curtisii*
Acinetobacter baumannii	*Aeromonas hydrophila*	*Gardnerella vaginalis*
Staphylococcus aureus	*Moraxella atlantae*	*Salmonella enterica subsp. enterica ser. dublin (group D)*
Staphylococcus epidermidis	*Prevotella bivia*	*Streptococcus acidominus*
Shigella sonnei	*Pasteurella aerogenes*	

**Microorganisms evaluated as extracted DNA were tested in copies/ml.*

f. Interfering Studies

This study was conducted to evaluate the potential interference of a panel of 27

endogenous and exogenous substances with the Panther Fusion GBS Assay (**Table 11**). Potentially interfering substances were added to clinical negative Lim Broth matrix with or without GBS spiked at 3X LoD. No interference with the detection of GBS was observed with the panel of endogenous/exogenous substances. Results of the Interference Study are acceptable.

Table 11. List of Potentially Interfering Substances Tested

Substance Name	Concentration
Human Amniotic Fluid	4% v/v
Human Whole Blood EDTA	4% v/v
Human Whole Blood Na Citrate	4% v/v
Human Serum	4% v/v
Human Urine Sample	4% v/v
Human Fecal Sample	4% v/v
Topical Hemorrhoid Ointment (Preparation H Cream)	3.4% w/v
Anti-Diarrheal Medication (Pepto Bismol)	4% v/v
Personal Lubricant (K-Y Jelly Personal Lubricant)	2.2% w/v
Lubricating gel (Aquagel)	2.1% w/v
Vaginal Anti-itch Cream (OTC) (Vagisil)	3.9% w/v
Vaginal Anti-itch Cream (OTC) (Gyno-Daktarin)	3.8% w/v
Vaginal Antifungal Cream (OTC) (Monistat)	3.1% w/v
Vaginal Antifungal Gel	3.0% w/v
Anti-Diarrheal Caplet (Kaopectate)	1.1% w/v
Deodorant Powder (Vagisil)	1.1% w/v
Deodorant Suppositories (Norforms Suppositories)	2.1% w/v
Deodorant Spray (FDS)	1.5% w/v
Body Powder (Gold Bond Powder)	0.4% w/v
Body Oil	4% v/v
Spermicidal Foam	2.1% w/v
Oral Laxative (Metamucil Fiber Supplement)	2.2% w/v
Grains de Vals (SennosideB)	0.4% w/v
Oral Laxative (Phillips Milk of Magnesia)	7.3% w/v

Substance Name	Concentration
Stool Softener	0.9% w/v
Astroglide Liquid Personal Lubricant	2.7% w/v
Enema Solution (Fleet enema)	4% v/v

g. Carry-over/Cross Contamination

A Carry-Over and Cross Contamination Study for the Panther Fusion GBS Assay was conducted to verify that no cross-contamination occurs on the test system. Samples were tested with high positive samples (1 x 10^6 CFU/ml) and negative samples. Five runs of 60 reactions with alternating high positive (n=30) and negative (n=30) samples were processed on two Panther Fusion systems. A total of 7 runs were performed per instrument (14 total). All samples tested during the study gave the expected results. No carry-over/cross-contamination was observed.

h. Assay cut-off:

The RFU range threshold and Ct threshold results processing parameters were defined based upon the analysis of data generated during the development of the GBS assay. Data from 81 runs consisting of a mix of analytical and pre-clinical panels were used in the analysis. Samples with a GBS (FAM) Ct value less than the FAM Ct threshold of 40 are reported as GBS positive. Samples with a GBS (FAM) Ct value greater than the FAM Ct threshold of 40 and an IC (RED677) Ct value greater than the RED677 Ct threshold of 38 are reported as invalid.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

See Broth Equivalence Study above.

3. Clinical studies:

a. Clinical Sensitivity:

A prospective multicenter study was conducted at three geographically diverse sites using left-over enriched culture samples (Lim Broth and Carrot Broth) from vaginal/rectal swab specimens collected from antepartum women undergoing routine GBS screening. Vaginal/rectal specimen swabs were cultured in Lim Broth or Carrot Broth from 18-24 hrs. Of the total 959 specimens enrolled and tested during the

study, 12 Carrot Broth samples were removed from analysis as these did not meet the inclusion criteria (incubation time of enrichment exceeded 24 hrs).

A total of 947 specimens were compliant and provided valid results for evaluation from the three participating sites. Samples were tested with both the Reference Culture Method and the Panther Fusion GBS Assay to determine the clinical sensitivity and specificity. The Panther Fusion GBS Assay was performed according to the package insert. Ten invalids were observed (six hardware failures and four IC failures). All re-tests yielded a valid result. The study results are shown in **Table 12** below.

For all samples enriched in Lim Broth and those samples enriched in Carrot Broth for which no color change was observed, per the CDC algorithm, one blood agar plate (BAP) was inoculated for reference method testing. BAPs were incubated for up to 48 hours and inspected for organisms suggestive of GBS. The presence of GBS after incubation on BAP was confirmed by Gram staining, latex agglutination, and catalase tests. Carrot Broth cultures that changed to the expected color (indicative of GBS) were reported as GBS positive samples and not subcultured.

Table 12. Clinical Performance Data for Panther Fusion GBS Assay vs Reference Culture Method

Panther Fusion GBS Assay (Site)	**Number of Samples Tested**	**Panther Fusion GBS Assay Positives: Culture Positives**	**Sensitivity % [TP/(TP +FN)][a]**	**Panther Fusion GBS Assay Negatives: Culture Negatives**	**Specificity % [TN/(FP + TN)][b, c]**
Lim Broth (Site A)	300	65:60	60/60 = 100% (95% CI: 93.98% - 100%)	235:240	235/240 = 97.92% (95% CI: 95.22% - 99.11%)
Lim Broth (Site B)	343	71:60	60/60 = 100% (95% CI: 93.98% - 100%)	272:283	272/283 = 96.11% (95% CI: 93.18% - 97.82%)
Total Lim Broth (Site A & Site B)	643	136:120	120/120 = 100% (95% CI: 96.90% - 100%)	507:523	507/523 = 96.94% (95% CI: 95.09% - 98.11%)
Carrot Broth (Site C)	304	93:83	83/83 = 100% (95% CI: 95.58% - 100%)	211:221	211/221 = 95.47% (95% CI: 91.87% - 97.52%)
Combined	*947*	*229:203*	*203/203 = **100%** (95% CI: 98.14% - 100%)*	*718:744*	*718/744 = **96.50%** (95% CI: 94.93% - 97.60%)*

[a] *TP= true positive, FN= false negative*

[b] *TN=true negative, FP= false positive*

[c] *Of 16 FPs with Lim Broth, 14 (87.5%) were positive by another FDA cleared GBS nucleic acid test. The 10 FPs with Carrot broth were not tested with another FDA cleared GBS nucleic acid test.*

b. Clinical specificity:

See above

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The performance of the Panther Fusion GBS Assay was evaluated in a three-site prospective Clinical Study in the U.S. on enriched Lim Broth and Carrot Broth cultures of vaginal/rectal swabs from antepartum women. Overall, the percent positivity for GBS as determined by the Panther Fusion GBS Assay was 21.2% and 30.6% for Lim Broth and Carrot Broth, respectively. By the Reference Culture Method, the prevalence of GBS was 18.7% and 27.3% using Lim Broth and Carrot Broth, respectively.

N. Instrument Name:

Panther Fusion System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Specimen is labeled with a unique barcode, which is tracked by the software to prevent re-use and for positive sample identification.

4. Specimen Sampling and Handling:

   Prior to testing on the Panther Fusion system, resuspend the enriched specimen and

transfer 1 ml of the specimen to the Aptima Specimen Transfer Tube containing 2.9 ml of Specimen Transport Medium.

5. Calibration:

   Real Time Fluorometers (RTF) undergo a single calibration during manufacturing. No additional calibration is performed by the end user.

6. Quality Control:

   Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and a laboratory's standard quality control procedures.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.